

US serial No. 09/851,084
Applicant's docket No. Oddy 001

--IN THE CLAIMS--

This listing of claims will replace all prior versions, and listings, of claims in the application:

1.(currently amended) A method of expressing PCA (protein complementation assays) interacting protein partners in plant material comprising:

(A) transforming said plant material with:

(1) a first partner construct coding for a first fusion product comprising:

(a) a first fragment of a first ~~protein~~ enzyme whose fragments can exhibit a detectable enzymatic activity when associated and

(b) a first protein-protein interacting domain; and

(2) a second partner construct coding for a second fusion product comprising:

(a) a second fragment of said first ~~protein~~ enzyme and

(b) a second protein-protein interacting domain that can bind (1)(b);

(B) culturing said material under conditions allowing expression of said PCA interacting protein partners, and allowing interaction of said first interacting domain with said second interacting domain;

(C) directly or indirectly testing for reconstitution of said enzymatic activity when said ~~protein~~ enzyme fragments are associated.

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2. (previously amended) The method of claim 1 wherein said plant material is selected from the group consisting of whole plants and plant-derived organs, tissues, cells, subcellular parts, and protoplasts.

3. (previously amended) The method of claim 1 or claim 2 wherein said plant material is derived from a transgenic plant.

4. (currently amended) The method of claim 1 wherein an inducer is added in step (B) to facilitate the interaction of said first protein-protein interacting domain and said second protein-protein interacting domain, wherein culturing with said inducer increases the level of said enzymatically detectable product over the level detectable when culturing without said inducer under otherwise identical conditions.

5. (currently amended) The method of claim 1 or 4 wherein said enzymatically detectable product is a detectable fluorescent product and wherein said detection means is selected from the group consisting of fluorescence microscopy, spectrofluorometry, FACS analysis, and a fluorescence-detecting video system.

6. (previously amended) The method of claim 1 or 4 wherein said plant material is cultured on a medium selective for the enzymatic activity of said enzyme reporter molecule.

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Claims 7-26 (withdrawn)

Claim 27. (previously added) The method of claim 1 wherein said first and second protein fragments which exhibit activity when associated are derived from dihydrofolate reductase (DHFR) and wherein said plant material is cultured on medium selective for DHFR activity.

Claim 28. (previously added) The method of claim 1 wherein said transforming comprises a method selected from the group consisting of electroporation and vacuum infiltration.

Claim 29. (previously added) The method of claim 4 wherein said first and second protein fragments which exhibit activity when associated are derived from dihydrofolate reductase (DHFR) and wherein said plant material is cultured on medium selective for DHFR activity.

Claim 30. (previously added) The method of claim 4 wherein said inducer is selected from the group consisting of rapamycin and salicylic acid.

Claim 31. (currently amended) A method of expressing PCA (protein fragment complementation assays) interacting protein partners in plant material comprising:

(A) transforming said material with:

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(1) a first construct coding for a first fusion product comprising:

- (a) a first fragment of a first enzyme reporter whose fragments can exhibit an enzymatically detectable activity when associated and
- (b) a first protein-protein interacting domain; and

(2) a second construct coding for a second fusion product comprising:

- (a) a second fragment of said first enzyme reporter and
- (b) a second protein-protein interacting domain that can bind (1)(b)

wherein said interacting protein partners are comprised of a first partner and a second partner comprised of said first fusion product and said second fusion product respectively, and wherein said first fusion product and said second fusion product are able to interact and associate to reconstitute an active enzyme reporter molecule;

(B) culturing said material under conditions allowing expression of said PCA interacting protein partners and allowing interaction of said first interacting domain with said second interacting domain, wherein said conditions include the presence of a substrate for said active enzyme reporter molecule and wherein said substrate forms a fluorescent detectable product when acted upon by said active enzyme reporter molecule; and

(C) detecting said fluorescent detectable product by a detection means capable of detecting said fluorescent detectable product.

Claim 32. (currently amended) A method of expressing PCA (protein fragment complementation assays) interacting protein partners in plant material comprising:

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(A) transforming said material with:

(1) a first construct coding for a first fusion product comprising

(a) a first fragment of a first molecule and

(b) a first protein-protein interacting domain; and

(2) a second construct coding for a second fusion product comprising

(a) a second fragment of said first molecule and

(b) a second protein-protein interacting domain that can bind (1)(b)

wherein said interacting protein partners are comprised of a first partner and a second partner comprised of said first fusion product and said second fusion product respectively, and wherein said first fusion product and said second fusion product are able to interact and associate to form an enzymatically active molecule;

(B) culturing said material under conditions allowing expression of said PCA interacting protein partners and allowing interaction of said first interacting domain with said second interacting domain, wherein said conditions include the presence of a substrate for said enzymatically active molecule and wherein said substrate forms a detectable fluorescent product when acted upon by said enzymatically active molecule; and

(C) detecting said fluorescent product by a detection means capable of detecting said fluorescent product, wherein said first molecule is a dihydrofolate reductase molecule (DHFR).

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Claim 33. (previously presented) The method of claim 32 wherein an inducer is added in step (B) to facilitate the interaction of said first protein-protein interacting domain and said second protein-protein interacting domain and wherein culturing with said inducer increases the level of detectable fluorescence from said fluorescent product over the level detectable when culturing without said inducer under otherwise identical conditions.